Collagen synthesis activity in the aqueous humour of eyes with glaucoma surgery: a pilot study

Anja Tuulonen, Juha Risteli, Leila Risteli, Juha Välimäki, P Juhani Airaksinen

Abstract

Aims—The purpose of this pilot study was to test whether the rate of collagen synthesis is measurable in the aqueous humour samples in reoperated and previously unoperated eyes.

Methods—The material consisted of 28 eyes of 27 patients, aged 5 to 82 years, in whom aqueous humour samples were obtained during eye surgery. Fifteen patients had no history of previous eye surgery (control group) while 12 patients were re-operated (study group). The carboxyterminal propeptide of type I procollagen (PICP) and the aminoterminal propeptide of type III procollagen (PIIIINP) were measured by specific immunoassays in the aqueous humour samples.

Results—The mean concentration of PIIIINP in the study group (8·4 (SD 12·5) μg/l) was statistically significantly larger than that of the control group (0·4 (0·4) μg/l) (p < 0·003?). The respective values for PICP were 98·8 (SD 177·7) μg/l in the study group and 0·7 (SD 2·8) μg/l in the control group (p = 0·0005). The eyes in the study group which were re-operated within 1 year showed values increased 20-fold compared with the eyes in the control group and those eyes in the study group which had had their previous operation more than a year ago. In three eyes aqueous humour samples were also obtained from the encapsulated Molteno bleb and showed values increased 12-fold compared with those from the anterior chamber.

Conclusions—PICP and PIIIINP immunoassays are suitable for measuring the rate of collagen synthesis in the aqueous humour and may be useful in studies on pharmacological modulation of wound healing in glaucoma surgery.

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Excessive fibroproliferation after glaucoma surgery leads to dense collagenous scar formation and filtration failure.1 2 The wound healing itself as well as its pharmacological modulation are intricate processes3 4 which have gained vast clinical significance with the increased use of antineoplastic agents, most often mitomycin C and 5-fluorouracil.5 6 The complexity of these processes in glaucoma surgery is especially emphasised since the filtration wound consists of eight elements7 which are expected to heal selectively and is perfused by the aqueous humour whose possible role in regulating the production of the episcleral bleb formation has long been recognised.8 9 Most reports of the wound healing process and its modulation have concentrated on studying prevention of fibroblast proliferation in tissue cultures and animal models. To our knowledge there are no human studies in which an attempt has been made to measure the production of new extracellular matrix components—for example, collagen and glycosaminoglycans—in response to filtering surgery. Fibrillar collagens are synthesised from procollagens which contain propeptide domains at both ends. When these are cleaved off, their concentrations can be measured by immunoassay methods and are directly related to the rate of collagen synthesis.10 14 The propeptides have been studied extensively in the wound fluid and serum after abdominal surgery.15 16

The purpose of this pilot study was to test whether it is possible to find measurable amounts of procollagen propeptides in aqueous humour samples. Since the history of previous eye surgery is known to increase the risk of filtration failure17 18 we wanted to study differences in the collagen synthesis activity between previously unoperated eyes and eyes undergoing reoperation.

Material and methods

The material consisted of 28 eyes of 27 patients operated by two ophthalmic surgeons (AT and PJA). Fifteen phakic eyes of 15 patients had no history of previous eye surgery (control group) while 13 eyes of 12 patients underwent re-operation (study group) (Tables 1 and 2). Three of the 13 eyes were pseudophakic and 10 were phakic. The eyes had been operated one to four (mean two) times before this study. No antimetabolites were used.

In the surgical technique, the conjunctiva was excised first, followed by the corneoscleral groove (in extracapsular cataract extraction), or cutting of the scleral flap (in trabeculotomy), and suturing the implant to the sclera (in the Molteno operation). Thereafter, aqueous humour samples (≈0·1 ml) were obtained from the anterior chamber with a 25 gauge needle which was the first instrument to enter the eye. In three eyes an aqueous humour sample was also obtained from an encapsulated Molteno bleb. These samples were drawn with a needle through the encapsulated bleb wall around the plate after it was exposed from the conjunctiva. Informed consent was obtained from all patients after the nature and
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Table 1 Description and procollagen propeptide concentrations (μg/l) of the control group (no history of previous eye surgery)

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Diagnosis</th>
<th>Age (years)</th>
<th>Procedure</th>
<th>PIIINP</th>
<th>PICP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cataract and exfoliative glaucoma</td>
<td>77</td>
<td>ECCE* and trabeculectomy</td>
<td>0.3</td>
<td>ND†</td>
</tr>
<tr>
<td>2</td>
<td>Cataract</td>
<td>78</td>
<td>ECCE</td>
<td>0.1</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>Cataract with exfoliation, no glaucoma</td>
<td>80</td>
<td>ECCE</td>
<td>0.5</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>Cataract</td>
<td>51</td>
<td>ECCE</td>
<td>0.2</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Cataract with exfoliation, no glaucoma</td>
<td>75</td>
<td>ECCE</td>
<td>0.5</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>Cataract</td>
<td>79</td>
<td>ECCE</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>Cataract</td>
<td>72</td>
<td>ECCE</td>
<td>0.2</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>Cataract and primary open angle glaucoma</td>
<td>75</td>
<td>ECCE</td>
<td>0.5</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>Cataract</td>
<td>81</td>
<td>ECCE</td>
<td>0.5</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>Cataract, diabetic maculopathy</td>
<td>67</td>
<td>ECCE</td>
<td>1.4</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>Cataract</td>
<td>58</td>
<td>ECCE</td>
<td>0.4</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>Cataract</td>
<td>70</td>
<td>ECCE</td>
<td>0.3</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>Uveitic glaucoma</td>
<td>42</td>
<td>Molteno bleb (1)</td>
<td>0.8</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>Low tension glaucoma</td>
<td>80</td>
<td>Trabeculectomy</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>Cataract and primary open angle glaucoma</td>
<td>82</td>
<td>ECCE and trabeculectomy</td>
<td>0.5</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ECCE=extracapsular cataract extraction, ND=not detected.
PPIINP=aminoterminal propeptide of type III procollagen, PICP=carboxyterminal propeptide of type I procollagen.

Results

The ages of the patients ranged from 5 to 82 years. The patients in the study group were younger than those of the control group (p<0.001) (Tables 1 and 2). The concentrations of both PIIINP and PICP (μg/l) were statistically significantly higher in the study group than in the control group (p<0.0037 and <0.0005, respectively) (Tables 1 and 2). The peak values were measured to 2 months after surgery in the study group.

Table 2 Description and procollagen propeptide concentrations (μg/l) of eyes operated previously

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Glaucoma diagnosis</th>
<th>Age (years)</th>
<th>Procedure (No of previous operations)</th>
<th>PIIINP†</th>
<th>PICP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rieger's anomaly</td>
<td>5</td>
<td>Revision of encapsulated Molteno bleb (1)</td>
<td>2.6</td>
<td>28.8</td>
</tr>
<tr>
<td>2</td>
<td>Pigmentary glaucoma</td>
<td>40</td>
<td>Revision of encapsulated Molteno bleb (2)</td>
<td>2.5</td>
<td>17.0</td>
</tr>
<tr>
<td>3</td>
<td>Angle recession</td>
<td>8</td>
<td>Revision of encapsulated Molteno bleb (2)</td>
<td>28.3</td>
<td>592.0</td>
</tr>
<tr>
<td>4</td>
<td>Rieger's syndrome, cornea plana</td>
<td>25</td>
<td>Molteno bleb (1)</td>
<td>27.9</td>
<td>259.0</td>
</tr>
<tr>
<td>5</td>
<td>Congenital</td>
<td>19</td>
<td>Re-Molteno (4)</td>
<td>2.4</td>
<td>(165.0)</td>
</tr>
<tr>
<td>6</td>
<td>Exfoliative, pseudophakia</td>
<td>71</td>
<td>Molteno bleb (3)</td>
<td>31.0</td>
<td>210.0</td>
</tr>
<tr>
<td>7</td>
<td>Neovascular</td>
<td>27</td>
<td>Revision of encapsulated Molteno bleb (3)</td>
<td>3.3</td>
<td>26.1</td>
</tr>
<tr>
<td>8</td>
<td>Primary open angle</td>
<td>85</td>
<td>Molteno (2)</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td>9</td>
<td>Exfoliative, pseudophakia</td>
<td>73</td>
<td>ECCE (2)</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>Uveitic, pseudophakia</td>
<td>48</td>
<td>Molteno (3)</td>
<td>0.8</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>Exfoliative</td>
<td>66</td>
<td>Molteno (1)</td>
<td>0.3</td>
<td>28.3</td>
</tr>
<tr>
<td>12</td>
<td>Primary open angle</td>
<td>69</td>
<td>Trabeculectomy (1)</td>
<td>ND§</td>
<td>5.3</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>44</td>
<td>Retrabeculectomy (1)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Time refers to the interval between the last two surgical interventions in months. †The values in parenthesis are those obtained from encapsulated Molteno blebs. ‡Because of extremely shallow anterior chamber due to cornea plana, no sample was taken from the anterior chamber. §ND=not detected.
PPIINP=aminoterminal propeptide of type III procollagen, PICP=carboxyterminal propeptide of type I procollagen.
more than 20-fold higher PIINP values (mean 14·0 (SD 14·1) μg/l) than the eyes which had had their previous operation more than a year ago (mean 0·64 (0·61) μg/l), or the eyes in the control group (0·4 (0·4) μg/l). The PICP values in some eyes of the study group were high even 3 to 4 years after first surgery (Table 2).

One patient with Rieger’s anomaly (patient no 1, left eye in Table 2) underwent three surgical interventions: (1) Molteno operation, (2) revision of the encapsulated bleb at 1 month postoperatively with an uncontrollable pressure of 50 mm Hg, and (3) a second Molteno implant was inserted 1 month later as the first tube got buried in the cornea after progressing peripheral synechiae formation. The aqueous humour samples were obtained during the second and third operations. The sample acquired in the third operation showed threefold higher concentrations of the procollagen propeptides (PIINP 8·4 μg/l and PICP 46·5 μg/l), than those obtained during the second operation.

In three eyes aqueous samples were obtained both from the anterior chamber and from the Molteno bleb. The aqueous from the bleb showed 12-fold greater values compared with those of the anterior chamber (Tables 1 and 2).

Discussion

The results of this pilot study show that immunoassays of the procollagen propeptides PICP and PIINP can be used to measure collagen synthesis activity in the aqueous humour after glaucoma surgery and thus be applicable in in vivo studies of pharmacological modulation of the wound healing process. Rabbits are not suitable as animal models because the assays use antibodies prepared in rabbits. The activity of collagen synthesis in the aqueous humour was very high in some recently operated eyes but there were large inter-individual differences, which require further investigation – for example, the effect of glaucoma medications on filtration failure needs to be studied in a larger number of patients. Caution must be exercised in interpreting the results of this pilot study because of the limited numbers, the lack of randomisation, the heterogeneity of the clinical disorders and the gap in getting samples between 3 and 10 months postoperatively.

One might argue that the raised findings in recently operated eyes could be the result of artefacts, such as leakage from a defective blood-aqueous barrier, or from vessels around the bleb area. This is not probable, however, since the propeptide ratio (PICP:PIINP) in our samples is lower than in serum suggesting a reflection of local tissue reaction rather than serum leakage. In addition, leakage of blood from the bleb area is not likely since the bleb wall, through which the sample is drawn, is a totally avascular, thick fibros membrane impermeable even to aqueous humour.

Molteno reported that after an initial hypotensive stage, the Molteno operated eyes were hypertensive approximately 1 to 3 months after surgery, exactly at the time of peak collagen synthesis activity measured in the present study. The use of systemic corticosteroids resulted in a thinner bleb and lower final intraocular pressure. Corticosteroids inhibit the inflammatory response, and also fibroblast proliferation, when given in appropriate concentrations. In addition, they repress the synthesis of types I and III procollagens. Although corticosteroids are widely used after filtration surgery, they have received surprisingly little attention in the literature of clinical trials, in comparison with the vast number of papers on antineoplastic agents.

In terms of controlling the wound healing process, implant surgery differs from trabeculectomy. If the surgical site is scarred in trabeculectomy, it is not probable that the fistula could be later opened with any pharmacological modulators of the wound healing process. However, in implant surgery the fistula is maintained by the tube and it is possible to affect the scarring process also at a later stage. In order to avoid the problems related to the early use of present wound healing modulators, it would be more physiological to allow the wound healing process to start relatively undisturbed. The process could then be pharmacologically attacked later by thinning the dense collagenous tissue around the implant plate, preferably during the maximum activity of collagen synthesis which this study places at around 1 to 3 months after surgery. Further studies on a large number of patients are required to determine not only the optimum dosage and duration of treatment of the
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different wound healing modulators but also the most beneficial time of their application in the sequence of scar tissue formation.

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